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Signed R. Mahoney

Dated 8 April 2003

GB0206839.3

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of:-

CANCER RESEARCH TECHNOLOGY LIMITED
Incorporated in the United Kingdom
61 Lincoln's Inn Fields
London
WC2A 3PX
United Kingdom

ADP No. 08544520002

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

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P01/7700 0.00-0206839.3

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The Patent Office

Cardiff Road
Newport
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NP10 8QQ

1. Your reference

P013820GB ATM

2. Patent application number
(The Patent Office will fill in this part)

0206839.3

22 MAR 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Cancer Research Ventures Limited
5 Alfred Place
London
WC1E 7EB
United Kingdom

SECTION 30 (1977 ACT) APPLICATION FILED 07.03.03

Patents ADP number *(if you know it)*

7822440002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

Anti-Cancer Combinations

5. Name of your agent *(if you have one)*

D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

21 New Fetter Lane
London
EC4A 1DA

Patents ADP number *(if you know it)*

59006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
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Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

D Young & Co

Signature Date 22 March 2002

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom — Dr Malcolm Main — 023 8071 9500

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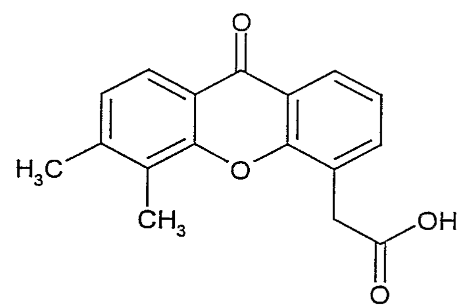
ANTI-CANCER COMBINATIONS

The present invention relates to synergistic combinations of the compounds of the class having the formula (I) as defined below, for example compounds of the xanthenone acetic acid class having the formula (II) as defined below, such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and non-steroidal anti-inflammatory drugs such as cyclo-oxygenase inhibitors, in particular diclofenac, which have anti-tumour activity. More particularly, the invention is concerned with the use of such combinations in the treatment of cancer and pharmaceutical compositions containing such combinations.

5,6-dimethylxanthenone-4-acetic acid (DMXAA) is represented by the following formula:



Phase I clinical trials of DMXAA have recently been completed, with dynamic MRI showing that it induces a significant reduction in tumour blood flow at well-tolerated doses. DMXAA is thus one of the first antivascular agents for which activity (irreversible inhibition of tumour blood flow) has been documented in human tumours. These findings are in agreement with preclinical studies using tumours or human tumour xenografts which showed that its antivascular activity produced prolonged inhibition of tumour blood flow leading to extensive regions of haemorrhagic necrosis.

Non-steroidal anti-inflammatory drugs (NSAIDs) share the capacity to suppress the signs and symptoms of inflammation. Many also exert antipyretic and analgesic effects. Salicylate is the major anti-inflammatory metabolite of aspirin, the original NSAID. Aspirin irreversibly acetylates and blocks platelet cyclooxygenase. Other NSAIDS are reversible inhibitors. Selectivity for COX-1 and COX-2 is variable for many of the traditional NSAIDs. Ibuprofen inhibits COX-2 and COX-1 to approximately the same

extent. However, highly selective COX-2 inhibitors (celecoxib and rofecoxib) are now available.

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) having the chemical name 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid. Diclofenac potassium is available as Cataflam® with Diclofenac sodium available as VOLTAREN®. Diclofenac is indicated for the acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis and treatment of ankylosing spondylitis, analgesia and primary dysmennorrhea.

Pharmacokinetic drug interaction is defined as one where drug A affects the plasma (or tissue) concentration of drug B, by altering the latter's absorption, distribution, excretion or metabolism (Dorr and Fritz, *Cancer Chemotherapy Handbook*, Henry Kimpton Publishers, London. 1980; Tenenbaum, L., Cancer chemotherapy - a Reference Guide, W. B. Saunders, New York. 1989). The combination therapy of 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and thalidomide is one of the examples of pharmacokinetic interactions that involve alteration in drug metabolism.

UGT lA9, UGT 2B7, and CYP 1A2 have been shown to be involved in the metabolism of DMXAA (Miners *et al* Cancer Res., 57: 284-289, 1997; Zhou *et al* J. Chromatog. B, 757: 343-348, 2001). Glucuronidation is the major metabolic elimination pathway of DMXAA (Miners *et al* Cancer Res., 57: 284-289, 1997; Kestell *et al*, Cancer Chemother. Pharmacol., 46: 135-141, 2000). DMXAA can also be metabolized by 6-methylhydroxylation, but to a lesser extent (Zhou *et al* J. Chromatog. B, 757: 343-348, 2001). The product of glucuronidation, DMXAA acyl glucuronide (DMXAA-G) , and the product of 6- methylhydroxylation, 6-methylhydroxyl-5-methylxanthenone-4-acetic acid (6-0H- MXAA), are then excreted in bile and urine (Zhou *et al* J. Chromatog. B, 757: 343-348, 2001).

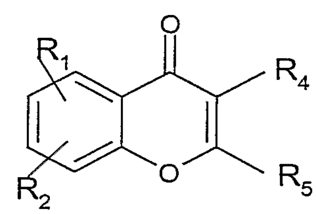
Diclofenac has been shown to affect the metabolism of DMXAA. At a concentration of 100μM, diclofenac has been shown to significantly inhibit glucoronidation (>70%) and 6-methylhydroxylation (>54%) of DMXAA in mouse and human microsomes. *In vivo*, diclofenac (100mg/kg i.p.) has been shown to result in a 24% and 31% increase in the plasma DMXAA AUC (area under the plasma concentration-time curve) and a threefold increase in $T_{1/2}$ ($P<0.05$) in male and female mice respectively (Zhou et al (2001) Cancer Chemother Pharmacol **47** 319-326).

It has now surprisingly been found that by administering, either concomitantly or sequentially, compounds having the formula (I) as defined below with an NSAID such as the NSAID diclofenac at NSAID concentrations which do not affect the plasma pharmacokinetics of compounds of formula (I), potentiation of the antitumour activity of compounds formula (I) as defined above is nevertheless achieved.

In particular co-administration of compounds of formula (I) as defined below such as DMXAA with NSAIDS such as diclofenac provides a therapeutic gain against sub-cutaneously established (3-5mm, approximately 20mg) colon 38 tumour fragments at concentrations of NSAID which does not significantly affect the plasma pharmacokinetics of the compound of formula (I) as defined below.

Thus, in a first aspect, the present invention provides a method for modulating neoplastic growth, which comprises administering to a mammal, including a human, in need of treatment an effective amount of a compound of the formula (I):

Formula (I)



or a pharmaceutically acceptable salt or ester thereof and concomitantly or sequentially administering an effective amount of a NSAID, wherein said effective amount of said NSAID is less than that required to substantially alter the plasma pharmacokinetics of the compound of the xanthenone acetic acid class having the formula (I) as defined above in said mammal;

wherein:

(a) R_4 and R_5 together with the carbon atoms to which they are joined, form a 6-membered aromatic ring having a substituent $-R_3$ and a radical –(B)-COOH where B is a linear or branched substituted or unsubstituted C_1-C_6 alkyl radical, which is saturated or ethylenically unsaturated, and wherein R_1, R_2 and R_3 are each independently selected from the group consisting of H, C_1-C_6 alkyl, halogen, CF_3, CN, NO_2, NH_2, OH, OR, NHCOR, $NHSO_2R$, SR, SO_2R or NHR, wherein each R is

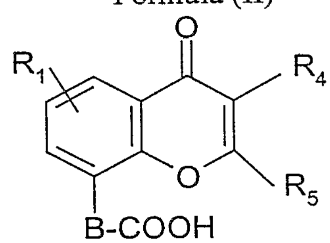
independently C_1 –C_6 alkyl optionally substituted with one or more substituents selected from hydroxy, amino and methoxy; or

(b) one of R_4 and R_5 is H or a phenyl radical, and the other of R_4 and R_5 is H or a phenyl radical which may optionally be substituted, thenyl, furyl, naphthyl, a C_1-C_6 alkyl, cycloalkyl, or aralkyl radical; R_1 is H or a C_1-C_6 alkyl or C_1-C_6 alkoxy radical; R_2 is the radical –(B)-COOH where B is a linear or branched substituted or unsubstituted C_1-C_6 alkyl radical, which is saturated or ethylenically unsaturated.

Where the radical –(B)-COOH is a substituted C_1-C_6 alkyl radical, the substituents may be alkyl, for example methyl, ethyl, propyl or isopropyl, or halide such as fluoro, chloro or bromo groups. A particularly preferred substituent is methyl.

In one embodiment of the first aspect of the invention, the compound of the formula (I) as defined above is a compound of the formula (II),

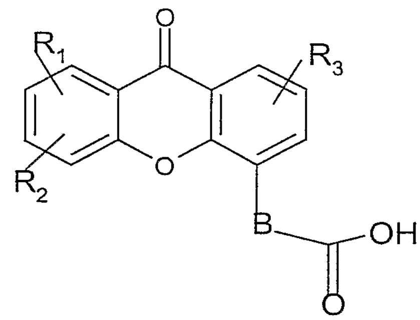
Formula (II)



where R_1, R_4, R_5 and B are as defined above for formula (I) in part (b).

In a preferred embodiment of the first aspect of the invention, the compound of formula (I) as defined above is a compound of the formula (III)

Formula (III)



wherein R_1, R_2 and R_3 are each independently selected from the group consisting of H, C_1-C_6 alkyl, halogen, CF_3, CN, NO_2, NH_2, OH, OR, NHCOR, $NHSO_2R$, SR, SO_2R or NHR, wherein each R is independently C_1 –C_6 alkyl optionally substituted with one or more substituents selected from hydroxy, amino and methoxy;

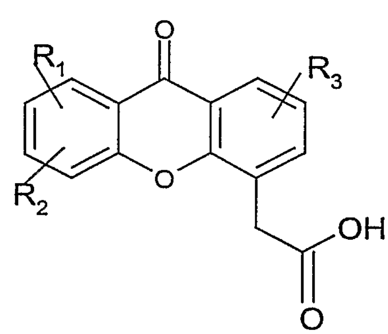
wherein B is as defined for formula (I) above;

and wherein in each of the carbocyclic aromatic rings in formula (I), up to two of the methine (-CH=) groups may be replaced by an aza (-N=) group;

and wherein any two of R_1, R_2 and R_3 may additionally together represent the group –CH=CH-CH=CH-, such that this group, together with the carbon or nitrogen atoms to which it is attached, forms a fused 6 membered aromatic ring.

Preferably, the compound of formula (III) is a compound of the formula (IV):

Formula (IV)

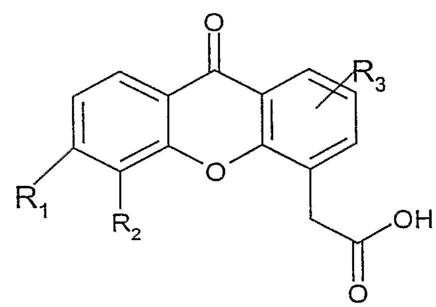


wherein R, R_1, R_2 and R_3 are as defined for formula (III).

In a preferred embodiment of the compound of formula (IV), R_2 is H, one of R_1 and R_3 is selected from the group consisting of C_1-C_6 alkyl, halogen, CF_3, CN, NO_2, NH_2, OH, OR, NHCOR, $NHSO_2R$, SR, SO_2R or NHR, wherein each R is independently C_1 –C_6 alkyl optionally substituted with one or more substituents selected from hydroxy, amino and methoxy, and the other of R_1 and R_3 is H.

Preferably, the compound of formula (IV) is of the formula (V):

Formula (V)



wherein R, R_1, R_2 and R_3 are as defined for formula IV.

Most preferably, the compound of formula (IV) is 5,6-dimethylxanthenone 4 acetic acid (DMXAA).

In the context of the present invention, a concentration of NSAID is considered not to substantially alter the plasma pharmacokinetics of the compound of formula (I) as defined above in the mammal if the plasma concentration of the compound of formula (I) in the mammal is not significantly increased (P<0.05), as judged by the compound of formula (I) AUC (area under the plasma concentration-time curve) and/or $T_{1/2}$ of the compound of formula (I) in plasma. Preferably neither the AUC nor the $T_{1/2}$ values are significantly different between mammals treated with the compound of formula (I) monotherapy and those treated with the compound of formula (I) and the NSAID. An alternative or preferably additional test to assess whether or not a concentration of NSAID may be considered in the context of the present invention to not substantially alter the plasma pharmacokinetics of a compound of formula (I) in a mammal may be measurement of metabolites. For example, where the compound of formula (I) is DMXAA, concentration of metabolites such as DMXAA acyl glucoronide (DMXAA-G) and 6-methylhydroxyl-5-methylxanthenone-4-acetic acid (6-OH-MXAA) may be

measured. A concentration of NSAID may be considered to not substantially alter the plasma pharmacokinetics of DMXAA in a mammal if the NSAID does not cause significant inhibition of glucoronidation or 6-methylhydroxylation of DMXAA as assessed by measurement of DMXAA –G or 6-OH-MXAA concentration in an assay of DMXAA metabolism in the presence and absence of the NSAID. Suitable in vitro and in vivo assays are known to the skilled person. For example, an in vitro assay based on liver microsomal preparations which may be used to assess DMXAA metabolism is described in Zhou et al (2001) Cancer Chemother Pharmacol **47** 319-326. More suitably, an High Performance Liquid Chromatography (HPLC) based method may be used to measure suitable HPLC based assay may be used to measure the concentrations of NSAID metabolites in the plasma or urine of a subject. Such an assay is described in Kestell,P et al (1999): Cancer Chemother. Pharmacol. 43, 323-330, the contents of which is hereby incorporated by reference.

In another aspect, the present invention provides the use of a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof for the manufacture of a medicament, for administration either concomitantly or sequentially with a unit dose of a cyclooxygenase inhibitor compound, for the modulation of neoplastic growth, wherein said unit dose comprises said NSAID compound in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in the mammal.

In a further aspect, the present invention provides the use of a NSAID compound for the manufacture of a unit dose of a medicament, for simultaneous, separate or sequential administration with a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof, for the modulation of neoplastic growth, wherein said unit dose comprises said NSAID compound in an amount which is less than that required to substantially alter the plasma pharmacokinetics of DMXAA in a subject to be treated.

In a still further aspect, the present invention provides a combined preparation of a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof and a NSAID compound for simultaneous, separate or sequential use, e.g. for modulation of neoplastic growth, wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID compound are present in a potentiating ratio, and wherein said NSAID compound is present in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to which the combination is administered.

In a further aspect, there is provided a pharmaceutical formulation comprising a combination of a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof and a NSAID compound wherein a unit dose of said pharmaceutical formulation comprises said NSAID compound in an amount which is less than that required to substantially alter the plasma pharmacokinetics of a compound of formula (I) as defined above in a subject to be treated.

The invention further provides a process for the preparation of a pharmaceutical formulation which process comprises bringing into association a combination of a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof and a NSAID compound with one or more pharmaceutically acceptable carriers therefor in a unit dose in which said NSAID compound is in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to be treated.

Furthermore, the invention also provides a kit comprising in combination for simultaneous, separate or sequential use in modulating neoplastic growth, a compound of formula (I) as defined above or a pharmaceutically acceptable salt or ester thereof and a NSAID compound, wherein said NSAID is provided in a unit dose comprising an amount of NSAID which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to be treated.

Brief Description of the Drawings

Fig. 1. shows growth of colon 38 tumours untreated (circle), or following treatment with DMXAA (25mg/kg, triangle), diclofenac (5mg/kg, diamond) or the combination (DMXAA (25mg/kg) and diclofenac (5mg/kg, square). Mean±SEM (standard error of the mean) of 5 mice per group.

Fig. 2 shows the time course of DMXAA plasma concentration following treatment with DMXAA(25mg/kg, circle), or the combination (DMXAA (25mg/kg) and diclofenac (5mg/kg, triangle). Mean±SEM of 3 mice per time point.

Fig. 3 shows the time course of DMXAA intratumoural concentration following treatment with DMXAA (25mg/kg, circle), or the combination (DMXAA (25mg/kg) and diclofenac (5mg/kg, triangle). Mean±SEM of 3 mice per time point.

In one embodiment the NSAID compound is a cylooxygenase inhibitor. Preferably the NSAID is a COX –2 inhibitor. In preferred embodiments of the invention, the NSAID is selected from the group comprising diclofenac, salicylate, ibuprofen, sulindac celecoxib and rofecoxib. In a particularly preferred embodiment, the NSAID is diclofenac.

The term 'potentiating ratio' is used herein to indicate that the compound of formula (I) as defined above or pharmaceutically acceptable salt or ester thereof and the NSAID compound are present in a ratio such that the antitumour activity of the combination is greater than that of either the compound of formula (I) or the NSAID compound alone or of the additive activity that would be predicted for the combinations based on the activities of the individual components. Thus the individual components act synergistically in combination provided they are present in a potentiating ratio.

The compound of formula (I) as defined above or pharmaceutically acceptable salt or ester thereof and the NSAID compound may be administered simultaneously, separately or sequentially. Preferably the compound of formula (I) as defined above or pharmaceutically acceptable salt or ester thereof and the NSAID compound are administered within 6 hours, more preferably 4 hours, more preferably 2 hours of one another. Most preferably the compound of formula (I) as defined above or pharmaceutically acceptable salt or ester thereof and the NSAID compound are administered simultaneously. For example the two drugs may be administered simultaneously by infusion over 0.2 to 6 hours, for example 0.33 to 3 hours.

Preferably the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID compound are administered in a potentiating ratio. Preferably the pharmaceutically acceptable salt is the sodium salt.

A potentiating ratio, for a compound of formula (I) as defined above and the NSAID which may be successfully used to treat cancer, is preferably in the range 150:1 to 1:15, more preferably in the range 75:1 to 1:10, even more preferably 50:1 to 1:5, for example 25:1 to 1:1, 15:1 to 1:1 . Suitably, the potentiating ratio is in the range 10:1 to 1:1. Most preferred is a potentiating ratio of approximately 5:1.

The amount of a combination of a compound of formula (I) or formula (II) as defined above, for example DMXAA or a pharmaceutically acceptable salt or ester thereof and the NSAID compound required to be effective as an anticancer agent will, of course, vary and is ultimately at the discretion of the medical practitioner. The factors to be considered include the route of administration and nature of the formulation, the

mammal's bodyweight, age and general condition and the nature and severity of the disease to be treated.

In general, a suitable effective dose of NSAID to be used in combination with DMXAA for administration to man for treatment of cancer may be a dose which is substantially non-toxic to man and which does not substantially affect the pharmacokinetics of DMXAA. A dose of NSAID may be considered to not substantially affect the pharmacokinetics of a compound e.g. DMXAA if, for example, it does not substantially inhibit glucoronidation or 6-methylhydroxylation of that compound, for example it inhibits glucoronidation or 6-methylhydroxylation of that compound by less than 40%, preferably less than 30%, 20%, 15%, 10%, 5%, 2%, most preferably less than 1% or 0.1%.

In general, a suitable effective dose of a combination of DMXAA and an NSAID for administration to man for treatment of cancer is in the range of 600 to 4900 mg/m^2 of DMXAA and 0.01 to 5 mg/kg of an NSAID such as diclofenac. For example from 600 to 4900 mg/m^2 of DMXAA and 0.025 to 4 mg/kg of an NSAID such as diclofenac, suitably 1200 to 3500 mg/m^2 of DMXAA and 0.05 to 4 mg/kg of NSAID, particularly 2000 to 3000 mg/m^2 of DMXAA and 0.1 to 3 mg/kg of NSAID, more particularly 2250 to 2750 mg/m^2 of DMXAA and 0.2 to 2.5 mg/kg of NSAID, more particularly 2250 to 2750 mg/m^2 of DMXAA and 0.05 to 2 mg/kg of NSAID. A particularly preferred dose is in the range 2250 to 2750 mg/m^2 of DMXAA and 0.75 to 1.25 mg/kg mg/m^2 of NSAID. A further particularly preferred dose is in the range 2250 to 2750 mg/m^2 of DMXAA and 0.1 to 0.5 mg/kg of NSAID, for example 0.1 to 0.25 mg/kg.

The compound of formula (I), or pharmaceutically acceptable salt or ester thereof and the NSAID compound may be administered in any suitable form. However, for use according to the present invention the combination of a compound of formula (I) or a pharmaceutically acceptable salt or ester thereof and a NSAID compound is preferably presented as a pharmaceutical formulation.

Pharmaceutical formulations comprise the active ingredients (that is, the combination of compound of formula (I) or a pharmaceutically acceptable salt or ester thereof and a NSAID compound) together with one or more pharmaceutically acceptable carriers therefor and optionally other therapeutic and/or prophylactic ingredients. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof.

Accordingly, the present invention provides a pharmaceutical formulation comprising a combination of compound of formula (I) or a pharmaceutically acceptable salt or ester thereof and a NSAID compound in association with one or more pharmaceutically acceptable carriers therefor, wherein the NSAID compound is present in an amount which is less than that required to substantially alter the plasma pharmacokinetics of compound of formula (I) in a subject to which the combination is administered..

The present invention further provides a process for the preparation of a pharmaceutical formulation which process comprises bringing into association a combination of compound of formula (I) or a pharmaceutically acceptable salt or ester thereof and a NSAID compound with one or more pharmaceutically acceptable carriers therefor, wherein said NSAID compound is present in said pharmaceutical formulation in an amount which is less than that required to substantially alter the plasma pharmacokinetics of compound of formula (I) in a subject to which the pharmaceutical formulation is administered..

Pharmaceutical formulations include those suitable for oral, topical (including dermal, buccal and sublingual), rectal and parenteral (including subcutaneous, intradermal, intramuscular and intravenous) administration as well as administration by naso-gastric tube. The formulation may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Preferably the pharmaceutical formulations are adapted for parenteral administration, most preferably intravenous administration. For example the compounds may be administered intravenously using formulations for each compound already known in the art.

Pharmaceutical formulations suitable for oral administration wherein the carrier is a solid are most preferably presented as unit dose formulations such as boluses, capsules or tablets each containing a predetermined amount of the active ingredients. A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compounds in a free-flowing form such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, lubricating agent, surface-active agent or dispersing agent. Moulded tablets may be made by moulding an inert liquid diluent.

Tablets may be optionally coated and, if uncoated, may optionally be scored. Capsules may be prepared by filling the active ingredients, either alone or in admixture with one or more accessory ingredients, into the capsule shells and then sealing them in the usual manner. Cachets are analogous to capsules wherein the active ingredients together with any accessory ingredient(s) are sealed in a rice paper envelope. The combination of compound of formula (I) or a pharmaceutically acceptable salt or ester thereof and a NSAID compound may also be formulated as dispersible granules, which may for example be suspended in water before administration, or sprinkled on food. The granules may be packaged e.g. in a sachet. Formulations suitable for oral administration wherein the carrier is a liquid may be presented as a solution or a suspension in an aqueous liquid or a non-aqueous liquid, or as an oil-in-water liquid emulsion.

Formulations for oral administration include controlled release dosage forms e.g. tablets wherein the active ingredients are formulated in an appropriate release - controlling matrix, or are coated with a suitable release - controlling film. Such formulations may be particularly convenient for prophylactic use.

The active ingredients may also be formulated as a solution or suspension suitable for administration via a naso-gastric tube.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Pharmaceutical formulations suitable for parenteral administration include sterile solutions or suspensions of the active combination in aqueous or oleaginous vehicles. Injectible preparations may be adapted for bolus injection or continuous infusion. Such preparations are conveniently presented in unit dose or multi-dose containers which are sealed after introduction of the formulation until required for use. Alternatively, the active ingredients may be in powder form which are constituted with a suitable vehicle, such as sterile, pyrogen-free water, before use.

The combination of compound of formula (I) or a pharmaceutically acceptable salt or ester thereof and NSAID compound may also be formulated as a long-acting depot preparation, which may be administered by intramuscular injection or by implantation e.g. subcutaneously or intramuscularly. Depot preparations may include, for example,

suitable polymeric or hydrophobic materials, or ion-exchange resins. Such long-acting formulations are particularly convenient for prophylactic use.

It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulations for the various routes of administration described above may include, as appropriate one or more additional carrier ingredients such as diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

Compounds of formula (I) and (II) may be prepared by methods known in the art. For instance, compounds of formula (I), wherein R_1, R_2, R_3, and R_4, are as defined in part (b) of the definition of formula (I) as recited above, may be prepared using the methods as disclosed in US 4,602,034 (Briet et al), the contents of which are herein incorporated by reference.

Compounds of formula (III), (IV) and (V) are known and may be prepared using the methods known in the art. For example, compounds of formula (III), (IV) and (V) and their preparation are described in the following references, the contents of which are herein incorporated by reference:

Rewcastle *et al*, Journal of Medicinal Chemistry 34(1): 217-22, January 1991;
Rewcastle *et al*, Journal of Medicinal Chemistry 34(2): 491-6, February 1991;
Atwell *et al*, Journal of Medicinal Chemistry 33(5): 1375-9, May 1990;
Rewcastle *et al*, Journal of Medicinal Chemistry 34(9): 2864-70, September 1991;
Rewcastle *et al*, Journal of Medicinal Chemistry 32(4): 793-9, April 1989

DMXAA may be prepared according to the methods described in Rewcastle *et al*, Journal of Medicinal Chemistry 34(1): 217-22, January 1991, the contents of which are incorporated herein by reference.

The NSAIDs may be prepared by any suitable method known to the skilled person. For example, diclofenac is a well known compound and can be prepared by methods known to those skilled in the art.

It is to be understood that the present invention covers all combinations of suitable and preferred groups described hereinabove.

The present invention will now be illustrated, but is not intended to be limited, by means of the following examples.

EXAMPLES

Materials and Methods

C57Bl/6 mice from the Animal Resource Unit, University of Auckland, were bred and housed under conditions of constant temperature and humidity, with sterile bedding and food, according to institutional ethical guidelines. All mice were aged between 8 and 12 weeks.

Drugs and Drug Administration

DMXAA was synthesized as the sodium salt (Rewcastle et al (1990) Journal of National Cancer Institute 82:528-529). DMXAA sodium salt was dissolved in sterile water and 25 mg/kg in a volume of 0.1 ml per 10 g body weight was injected intraperitoneally (i.p.) into mice.

Diclofenac (Sigma) was dissolved in dimethylsulphoxide, and was injected i.p. into mice in a volume of 25 μl per 10 g body weight. The required dose of diclofenac was injected concurrently with DMXAA.

Tumour Growth Delay Assay

Colon 38 tumour fragments (~1 mm^3) were implanted subcutaneously (s.c.) in the left flank of anaesthetized (sodium pentobarbital, 81 mg/kg) mice. The experiments were initiated when tumours were approximately 3 - 5 mm in diameter. Tumour-bearing mice were treated with drugs according to the administration schedule described before, and the tumours measured using calipers, three times weekly thereafter. Tumour volumes were calculated as $0.52a^2b$, where a and b are the minor and major axes of the tumour, respectively. The arithmetic means were calculated for each time point, counting cured tumours as zero volume. The growth delay was determined as the difference in the number of days required for the control versus treated tumours to increase four times in volume.

Pharmacokinetic Studies

DMXAA Sample Preparation

Mice were treated i.p. with DMXAA or DMXAA combination with diclofenac. At 0.25, 1.5, 3,4.5 and 6 hours after treatment, the mice were halothane- anaesthetised and the blood was collected through ocular sinus into heparinised plastic microcentrifuge tubes. The animals were then immediately killed by cervical dislocation. Tumour tissues were taken out immediately after mouse being killed, and stored at -70°C for later DMXAA assay.

Data Analysis and Assay Validation

For DMXAA pharmacokinetic studies, the AUC was calculated as a function of time using the log-trapezoidal rule. Cmax was the maximum concentration measured. The half-life ($T_{1/2}$) was calculated as 0.693/ Lz, where Lz is the slope of the terminal linear-portion of the log-concentration-time curve. The relative recoveries and coefficients of variation (CV) for the intra-assay accuracy and precision were 85 - 115 % and 6 - 10 % (n = 8 for plasma assay, and n = 10 for tumour/liver assay) respectively, over the concentration range of 0.2 - 100 μM (for DMXAA assay). Inter-assay accuracy was also acceptable with similar relative recoveries (85 - 115 %) and CVs (6 - 10 %, n =8 for plasma assay, and n = 10 for tumour/liver assay)

DMXAA Assay

DMXAA concentrations in plasma and in homogenates of tumour were measured using a specific reverse-phase high-pressure liquid chromatography (HPLC) assay. Automated solid-phase extraction and 2,5-dimethylxanthenone-4-acetic acid (as the internal standard) were used in this assay. Mouse plasma samples were centrifuged (6000 rpm, 5 min) (Biofuge A, Heraeus Christ GmbH, Germany), and then diluted 10-fold with 10 mM ammonium acetate buffer (pH 5.5). Tumour samples were homogenized in 1 ml of 10 mM ammonium acetate buffer (pH 5.5). Thereafter 200 μl of diluted plasma or tumour homogenates were mixed with the internal standard solution (50 μl, 20 μM), and proteins precipitated using ice-cold acetonitrile/methanol (3:1 v/v). After centrifugation (3000 rpm, 10 min, 4 °C), the supernatants were added to ammonium acetate buffer (9 ml) and transferred automatically onto 1 ml/ 100 mg preconditioned (1 ml acetonitrile/methanol. 3:1 v/v, and 1 ml Milli Q water) C18 Bond-Elut cartridges (Varian, Harbor City, California). This was accomplished using an automated sample preparation with an extraction column system (ASPEC, Gilson Medical, Middleton,

Wisconsin). The cartridges were washed with Milli Q water (1 ml) and the compounds of interest eluted using 1 ml acetonitrile containing 30 % methanol.

The elutes were evaporated to dryness using a centrifugal evaporator (Jouan, St. Nazaire, France) and the residues were dissolved in 200 μl mobile phase. Aliquots (18 μl) were automatically injected into the chromatograph (Waters WISP 712B sample injector and Model 510 pump; Water Associates, Milford, Massachusetts) with a fluorescence detector (Shimadzu Model RF530; Shimadzu, Kyoto, Japan) with excitation and emission wavelengths set at 345 and 409 nm, respectively, and a LUNA 5μ CI8(2) 100 X 4.6 mm stainless steel column (Phenomenex). Integration and data acquisition were achieved using a Unicam 4880 chromatography data system (Unicam, Cambridge, UK). Compounds were eluted from the column (retention time of DMXAA and internal standard were 8 and 6 minutes, respectively) using a mobile phase of 10 mM ammonium acetate buffer (pH 5.0) and acetonitrile (3:1, v/v) at a flow rate of 1.5 ml/min. Human plasma calibration samples were prepared by adding DMXAA to plasma over the concentration range 0.2 - 100 μM. Human plasma calibration samples were used. The peak-height ratios of DMXAA to the internal standard were plotted against DMXAA concentration in the calibration standards and the best fit straight line obtained by linear regression analysis. Quantitation of DMXAA in mouse plasma samples was achieved by determining the peak-height ratio in mouse plasma samples and using the equation obtained from the calibration curve.

Statistics

The statistical significance of tumour growth inhibition was tested by Students' *t*-test. Volumes of tumours were calculated using the formula 0.52 x minor axis squared x major axis.

Example 1- Tumour growth Delay

DMXAA (25 mg/kg) + diclofenac (5 mg/kg), a combination which was shown to be non-toxic in toxicity experiments in colon 38 tumour-bearing mice (results not shown), was compared with the DMXAA monotherapy against colon 38 tumours implanted s.c. in mice. The tumour growth delay experiment was conducted using 4 drug regimes: untreated controls, DMXAA alone (25 mg/kg), diclofenac alone (5 mg/kg), and a combination group of DMXAA (25 mg/kg) + diclofenac (5 mg/kg). The results are shown in Figure 1.

Diclofenac alone was found to have no significant effect on the growth of colon 38 tumours. DMXAA produced a growth delay of ~6 days, but none of the mice were cured. With the combination group, there was a remarkable improvement in the antitumour response in that all the mice were cured (100 %). The results showed that coadministration of diclofenac with DMXAA can lead to significant increases in antitumour activity.

Example 2 The Enhanced Antitumour Effect In the Presence Of Diclofenac Cannot Be Attributed to Pharmacokinetic Effects On The Metabolism Of DMXAA.

The effect of diclofenac on DMXAA' s plasma concentrations was next examined. The 3 hour time point was determined as being the best time point to use because of less variability (Dr Kestell, personal communication). The only combination that gave a significant increase (56 %) in DMXAA plasma concentration, was DMXAA (25 mg/kg) + diclofenac (I 00 mg/kg) (Table 1). All the doses of diclofenac below 100 mg/kg had no significant effect on DMXAA plasma concentrations 3 hours after administration.

An experiment was carried out to determine if diclofenac at 5 mg/kg had any effect on the plasma pharmacokinetics of DMXAA over the first 6 hours. No statistical difference in the DMXAA plasma concentrations were observed with or without coadministered diclofenac (Fig. 2). The AUC values, 1333 μM.hr and 1514 μM.hr, for DMXAA alone and in combination with diclofenac respectively, were also not statistically different. Similarly, the half-life of DMXAA in plasma (2.7 hours) was not statistically different from that obtained with coadministered diclofenac (3.6 hours). These results suggested that the reason for the observed improved antitumour activity with coadministered diclofenac was not as a result of alterations in the plasma pharmacokinetics of DMXAA.

Example 3 The Effect of Diclofenac on Intratumoral DMXAA

There was no significant difference in the DMXAA concentration in colon 38 tumours following treatment with DMXAA alone or in combination with diclofenac over a five hour time course (Figure 3). At six hours, however, there was a significant reduction in the DMXAA concentration. The AUC values, 507 μM.hr and 388 μM.hr, of DMXAA monotherapy and combination therapy respectively, showed no significant difference. The Cmax values, 111 μM and 100 μM, of DMXAA monotherapy and combination therapy respectively, similarly showed no significant difference. These results further indicate that the improved anti tumour activity in the presence of diclofenac is not attributable to altered pharmacokinetics of DMXAA.

Discussion

Diclofenac at high concentrations has been shown *in vitro* to inhibit glucuronidation (>70 %) and 6- methylhydroxylation (>54 %) of DMXAA (Zhou *et al*, Cancer Chemother. Pharmacol., 47: 319-326). *In vivo* diclofenac (100 mg/kg) is able to increase the plasma concentration and AUC of DMXAA by 24 - 31% in mice (Zhou *et al*, Cancer Chemother. Pharmacol., 47: 319-326). Diclofenac (100 mg/kg) increased DMXAA plasma concentration by 56 % (Table 5), but lower doses had no significant effect.

In this study, it had been shown that NSAIDS, in particular diclofenac at 5 mg/kg could enhance DMXAA anti tumour activity (Fig. 1). The growth delay for DMXAA monotherapy was around 6 days with no cure, whereas for DMXAA combination therapy, there was a significant increase in the number of cures. These results suggest that by coadministration of diclofenac, the anti tumour activity of DMXAA can be increased.

The time-course experiments (0 - 6 hours) of plasma and intratumoral concentrations in both DMXAA monotherapy and combination therapy were conducted (Fig. 2 and Fig. 3). Both of Cmax and AUC values induced by both treatments were similar. In this study, diclofenac was used at 5 mg/kg, a concentration significantly below the effective concentration that can inhibit glucuronidation of DMXAA. These results therefore suggest that the anti tumour activity enhanced by the dose combination of DMXAA (25 mg/kg) + diclofenac (5 mg/kg) was not due to the direct alteration in the pharmacokinetics of DMXAA.

Although there was no significant difference between two therapies in intratumoral AUC values, but there was a significant decrease at the 6 hour time point (Fig. 3), where the intratumoral concentration of DMXAA in the combination group was much lower than the one in the monotherapy. Without being bound by any one theory, it is possible that this might result from diclofenac enhancing DMXAA anti tumour activity by increasing the rate of reduction in tumor blood flow to the tumour, reducing the concentration of DMXAA inside the tumour tissue.

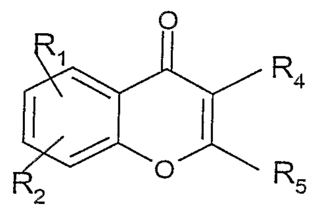
All publications mentioned in this specification are herein incorporated by reference. Various modifications and variations of the described methods and materials of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with

specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such embodiments. Indeed various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the following claims.

Claims

1. A method for modulating neoplastic growth, which comprises administering to a mammal, including a human, in need of treatment an effective amount of a compound of formula (I):

Formula (I)



or a pharmaceutically acceptable salt or ester thereof and concomitantly or sequentially administering an effective amount of a NSAID, wherein said effective amount of said NSAID is less than that required to substantially alter the plasma pharmacokinetics of the compound having the formula (I) as defined above in said mammal;

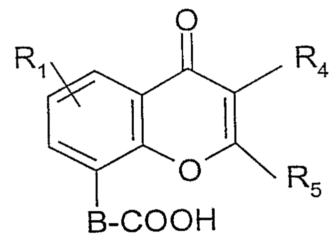
wherein:

(a) R_4 and R_5 together with the carbon atoms to which they are joined, form a 6-membered aromatic ring having a substituent $-R_3$ and a radical –(B)-COOH where B is a linear or branched substituted or unsubstituted C_1-C_6 alkyl radical, which is saturated or ethylenically unsaturated, and wherein R_1, R_2 and R_3 are each independently selected from the group consisting of H, C_1-C_6 alkyl, halogen, CF_3, CN, NO_2, NH_2, OH, OR, NHCOR, $NHSO_2R$, SR, SO_2R or NHR, wherein each R is independently C_1 –C_6 alkyl optionally substituted with one or more substituents selected from hydroxy, amino and methoxy; or

(b) one of R_4 and R_5 is H or a phenyl radical, and the other of R_4 and R_5 is H or a phenyl radical which may optionally be substituted, thenyl, furyl, naphthyl, a C_1-C_6 alkyl, cycloalkyl, or aralkyl radical; R_1 is H or a C_1-C_6 alkyl or C_1-C_6 alkoxy radical; R_2 is the radical –(B)-COOH where B is a linear or branched substituted or unsubstituted C_1-C_6 alkyl radical, which is saturated or ethylenically unsaturated.

2. The method according to claim 1 wherein the compound of Formula (I) is a compound of Formula (II):

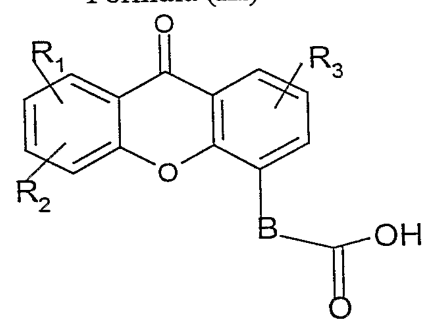
Formula (II)



where R_1, R_4, R_5 and B are as defined for formula (I) in claim 1 part (b).

3. The method according to claim 1 wherein the compound of Formula (I) is a compound of Formula (III):

Formula (III)



wherein R_1, R_2 and R_3 are each independently selected from the group consisting of H, C_1-C_6 alkyl, halogen, CF_3, CN, NO_2, NH_2, OH, OR, NHCOR, $NHSO_2R$, SR, SO_2R or NHR, wherein each R is independently C_1–C_6 alkyl optionally substituted with one or more substituents selected from hydroxy, amino and methoxy;

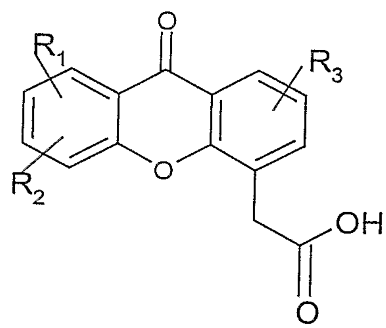
wherein B is as defined for formula (I) in claim 1;

and wherein in each of the carbocyclic aromatic rings in formula (I), up to two of the methine (-CH=) groups may be replaced by an aza (-N=) group;

and wherein any two of R_1, R_2 and R_3 may additionally together represent the group –CH=CH-CH=CH-, such that this group, together with the carbon or nitrogen atoms to which it is attached, forms a fused 6 membered aromatic ring.

4. The method according to claim 3, wherein the compound of Formula (I) is a compound of Formula (IV):

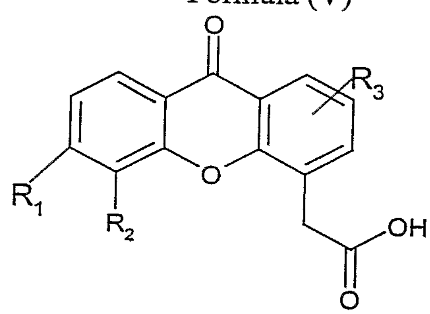
Formula (IV)



wherein R, R_1, R_2 and R_3 are as defined for formula (III) in claim 3.

5. A method according to claim 4 wherein the compound of Formula (IV) is a compound of formula (V):

Formula (V)



wherein R, R_1, R_2 and R_3 are as defined for formula IV in claim 4.

6. The method according to claim 1 wherein R_4 is H or a phenyl radical, R_5 is H or a phenyl radical which may optionally be substituted, thenyl, furyl, naphthyl, a C_1-C_6 alkyl, cycloalkyl, or aralkyl radical; R_1 is H or a C_1-C_6 alkyl or C_1-C_6 alkoxy radical; R_2 is radical –(B)-COOH where B is a linear or branched substituted or unsubstituted C_1-C_6 alkyl radical, which is saturated or ethylenically unsaturated.

7. A method according to any one of claims 1, 3, 4 or 5, wherein the compound of Formula (I) is DMXAA.

8. A method according to any one of the preceding claims wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID are administered in a potentiating ratio.

9. A method according claim any one of the preceding claims wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID are administered concomitantly.

10. A method according to any one of the preceding claims wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID are administered sequentially.

11. The method according to nay one of the preceding claims wherein the NSAID is a COX inhibitor.

12. The method according to any one of claims 1 to 10 wherein the NSAID is selected from the group comprising diclofenac, salicylate, ibuprofen, celecoxib and rofecoxib.

13. The method according to claim 12 wherein the NSAID is diclofenac.

14. The method according to any one of the preceding claims wherein the method is for modulation of neoplastic growth in colon cancer.

15. Use of a compound of formula (I) as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or ester thereof for the manufacture of a medicament, for simultaneous, separate or sequential administration with a unit dose of an NSAID compound, for the modulation of neoplastic growth, wherein said unit dose comprises said NSAID compound in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in the mammal.

16. Use of a NSAID compound for the manufacture of a unit dose of a medicament, for simultaneous, separate or sequential administration with a compound of formula (I) as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or ester thereof, for the modulation of neoplastic growth, wherein said unit dose comprises said NSAID compound in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to be treated.

17. Use according to claim 15 or claim 16 wherein the NSAID compound is selected from the group comprising diclofenac, salicylate, ibuprofen, celecoxib and rofecoxib.

18. The use according to claim 17 wherein the NSAID compound is diclofenac.

19. Use according to any one of claims 15 to 18 wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID compound are present in a potentiating ratio.

20. Use according to claim 19 wherein the ratio of compound of formula (I): NSAID is in the range 10:1 to 1:1.

21. Use according to claim 20 wherein the ratio of compound of formula (I): NSAID is about 5:1.

22. Use according to any of claims 15 to 21 wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID compound are administered concomitantly.

23. Use according to any of claims 15 to 21 wherein the compound of formula (I) or pharmaceutically acceptable salt or ester thereof and the NSAID compound are administered sequentially.

24. The use according to any one of claims 15 to 23 wherein the compound of formula (I) is DMXAA.

25. A pharmaceutical formulation comprising a combination of the compound of formula (I) as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or ester thereof and a NSAID compound wherein a unit dose of said pharmaceutical formulation comprises said NSAID compound in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to be treated.

26. A pharmaceutical formulation according to claim 25 wherein the formulation is adapted for intravenous administration.

27. A pharmaceutical formulation according to claim 25 or claim 26 wherein the NSAID compound is diclofenac.

28. A pharmaceutical formulation according to any one of claims 25 to 27 wherein the compound of formula (I) is DMXAA.

29. A process for the preparation of a pharmaceutical formulation which process comprises bringing into association a combination of a compound of formula (I) as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or ester thereof and a NSAID compound with one or more pharmaceutically acceptable carriers therefor in a unit dose in which said NSAID compound is in an amount which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to be treated.

30. The process according to claim 29 wherein the NSAID compound is diclofenac.

31. The process according to claim 29 or claim 30 wherein the compound of formula (I) is DMXAA.

32. A kit comprising in combination for simultaneous, separate or sequential use in modulating neoplastic growth, a compound of formula (I) as defined in any one of claims 1 to 6 or a pharmaceutically acceptable salt or ester thereof and a NSAID compound, wherein said NSAID is provided in a unit dose comprising an amount of NSAID which is less than that required to substantially alter the plasma pharmacokinetics of the compound of formula (I) in a subject to be treated

33. The kit according to claim 32 wherein the NSAID compound is diclofenac.

34. The kit according to claim 33 wherein the compound of formula (I) is DMXAA.

ABSTRACT

ANTI-CANCER COMBINATIONS

The present invention relates to synergistic combinations of the compounds of formula I such as compounds of the xanthenone acetic acid class such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and NSAIDs, in particular diclofenac, which have anti-tumour activity. More particularly, the invention is concerned with the use of such combinations in the treatment of cancer and pharmaceutical compositions containing said combinations.

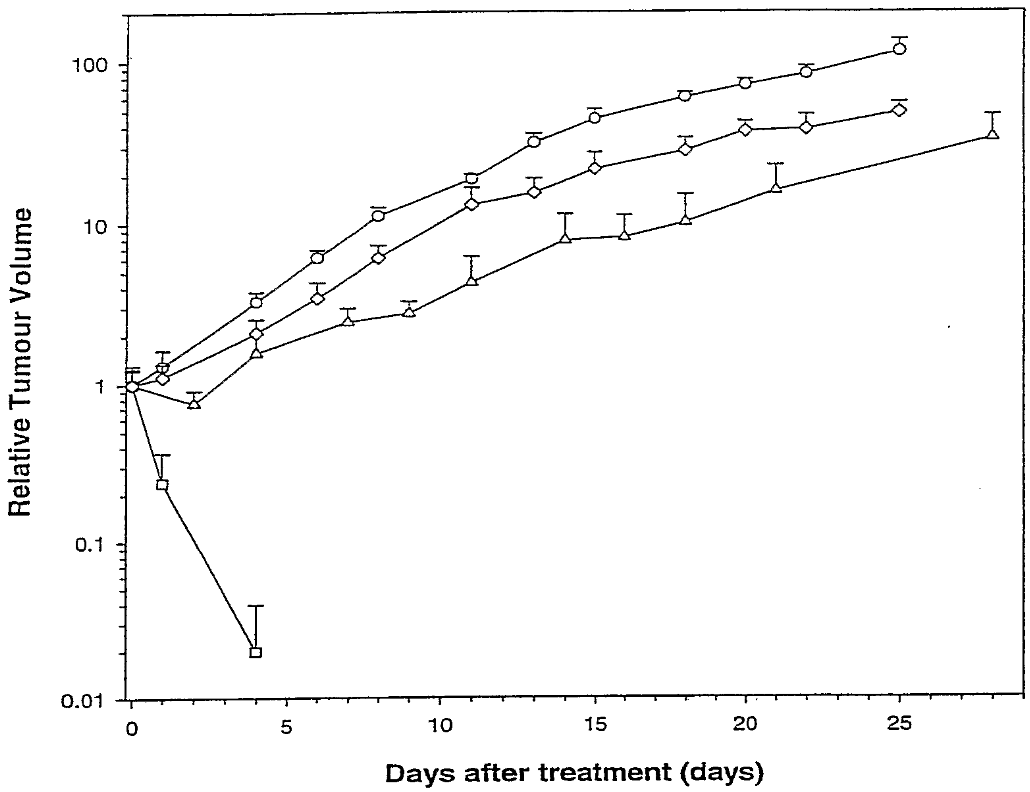


Figure 1 Growth of colon 38 tumours untreated (*circle*), or following treatment with DMXAA (25 mg/kg, *triangle*), diclofenac (5 mg/kg, *diamond*), or the combination (DMXAA (25 mg/kg) + diclofenac (5 mg/kg), *square*). Mean ± SEM of 5 mice per group.

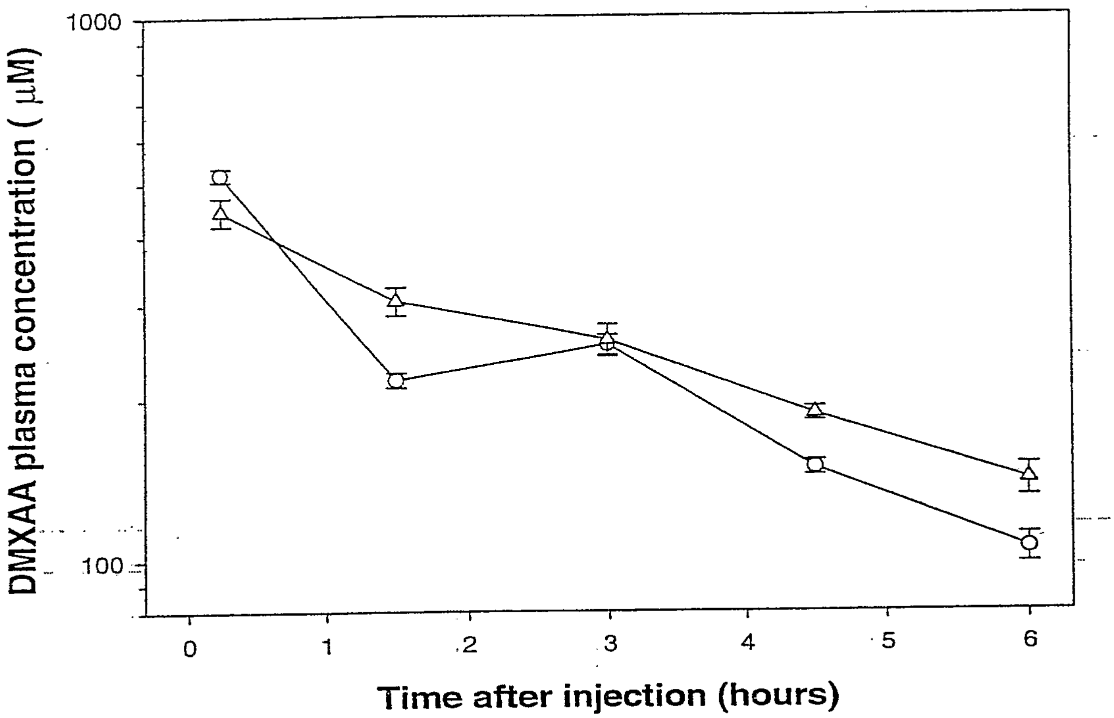


Figure 2 Time course of DMXAA plasma concentration following treatment with DMXAA (25 mg/kg, *circle*), or the combination (DMXAA (25 mg/kg) + diclofenac (5 mg/kg), *triangle*). Mean ± SEM of 3 mice per time point.

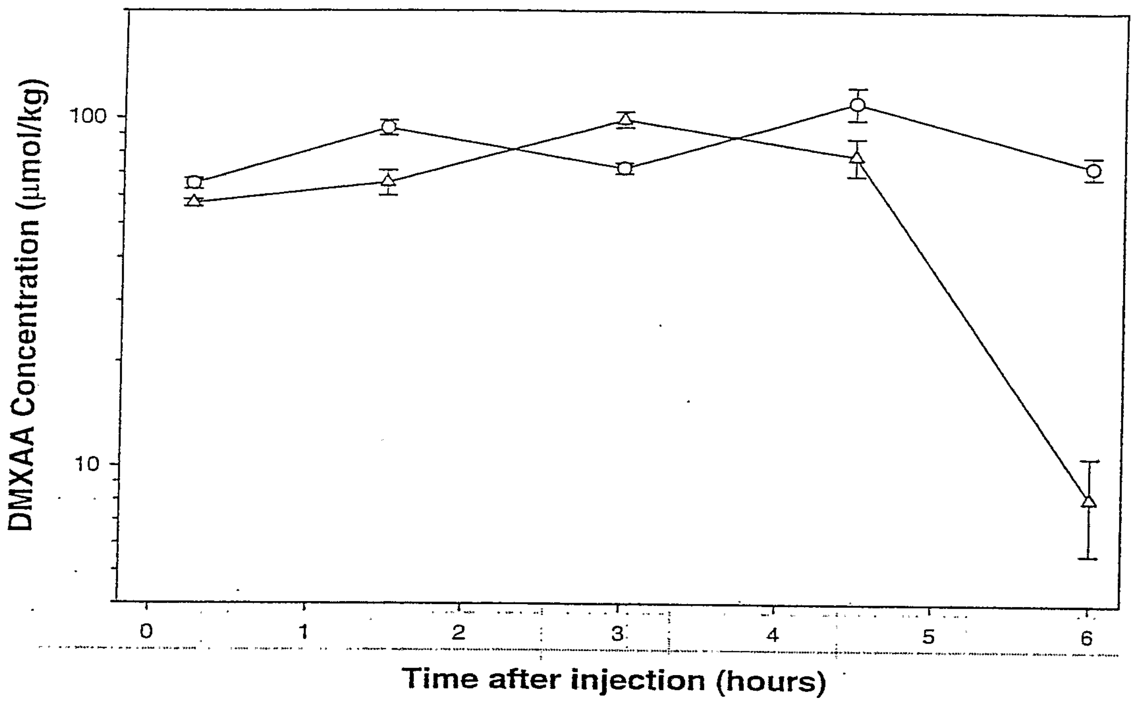


Figure 3 Time course of DMXAA intratumoral concentration following treatment with DMXAA (25 mg/kg, *circle*), or the combination (DMXAA (25 mg/kg) + diclofenac (5 mg/kg), *triangle*). Mean ± SEM of 3 mice per time point.